BIOMEDICAL AND ENVIRONMENTAL MASS SPECTROMETRY, 14, 295-299 (1987)

Short Communications

Dear Sir

A SYSTEM FOR COLLECTING HIGH-RESOLUTION TIME-OF-FLIGHT MASS SPECTROMETRIC DATA

We describe an 800,000 channel data system for ^{252}Cf fission fragment ionization time-of-flight mass spectrometry, and we give examples of some of the system's capabilities.

INTRODUCTION

We wished to develop a data system for a ^{252}Cf fission fragment ionization time-of-flight mass spectrometer which would allow us to use the instrument to its full capabilities. Such a data system should operate at a high rate of data acquisition to allow for high ion production rates. The dynamic range of the system should be great enough to allow the collection of data for long periods so that infrequently occurring ions can be observed without exceeding the capacity for abundant ions. The system's memory should be large enough to store an entire time-of-flight spectrum without degrading the intrinsic time resolution of the mass spectrometer.

In this paper we describe a data system, based on a Digital Equipment Corporation MicroVAX I 32-bit computer, which can store a spectrum comprised of as many as 800,000 5/8 ns channels covering a time span of 500 μs, has a dynamic range of $0\text{-}2\times10^9$ events per time channel, and can read and store flight times in periods on the order of tens of microseconds.

At present we know of two 32-bit data systems in use with the time-of-flight mass spectrometer (1,2). However the system we describe here is unique in its time range at high time resolution. Another convenient feature of the system is that it does not require special hardware to read and store the ion flight times since all of the data handling is done by software in the computer.

We demonstrate the capabilities of the data system by presenting mass spectrometric results obtained from horse heart myoglobin, human insulin, leu-enkephalin, and crystal violet.

EXPERIMENTAL

The Rockefeller University ^{252}Cf fission fragment ionization mass spectrometer has been described previously (3). In this instrument, the sample of interest, deposited in a thin layer on an aluminized mylar foil, is placed in front of a ^{252}Cf source. Fission fragments from the ^{252}Cf pass through the foil, causing the desorption of sample ions. These sample ions are accelerated through a potential difference of 10 kV. The ions are then allowed to drift through a 3 m long flight tube, at the end of which they are detected. When a ^{252}Cf fission fragment ionizes the sample, its complementary fragment strikes a detector to provide a time reference from which the flight times of the sample ions are measured by a time-to-digital converter (TDC). The TDC in our mass spectrometer has been described previously (4). It has a resolution of 5/8 ns and a time range of 1.3 ms with an integral non-linearity of less than 7 parts in 10^7. It can collect up to 15 different ion flight times in a given timing interval. In the system described here TDC control and data handling are done by a Digital Equipment Corporation MicroVAX I 32-bit computer using a

0887-6134/87/060295-05 $05.00

Received 25 February 1987

simple handshaking routine. The computer communicates with the TDC using two 16-bit ports on a (DEC) DRV11-J 64-line parallel interface. Digitized times from the TDC are 21 bits long, the least significant bit representing an interval of 5/8 ns. Each digitized time the computer receives corresponds to an element in an 800,000 word array in computer memory in which the time-of-flight spectrum is stored. When a time is received, the appropriate element in the array has its value increased by one. Each word in the time-of-flight array is a 32-bit signed integer word.

RESULTS AND DISCUSSION

a. Resolution: The large number of time channels in which a spectrum can be stored enables us to see fine details throughout the spectrum even when the spectrum extends to high mass (long flight times). This ability to observe fine details is illustrated in Figure 1, which shows a plot of the mass spectrum of horse heart myoglobin. Equine myoglobin is comprised of a peptide chain of 153 amino acid residues, called a globin, with an iron-protoporphyrin IX molecule noncovalently bound to it. The calculated isotopically averaged molecular weight (formula weight) for the globin portion of the molecule is 16,953 u. The calculated formula weight of iron-protoporphyrin IX using only light isotopes is 616.2 u. Our spectrum shows separate peaks for the globin and protoporphyrin portions of the molecule.

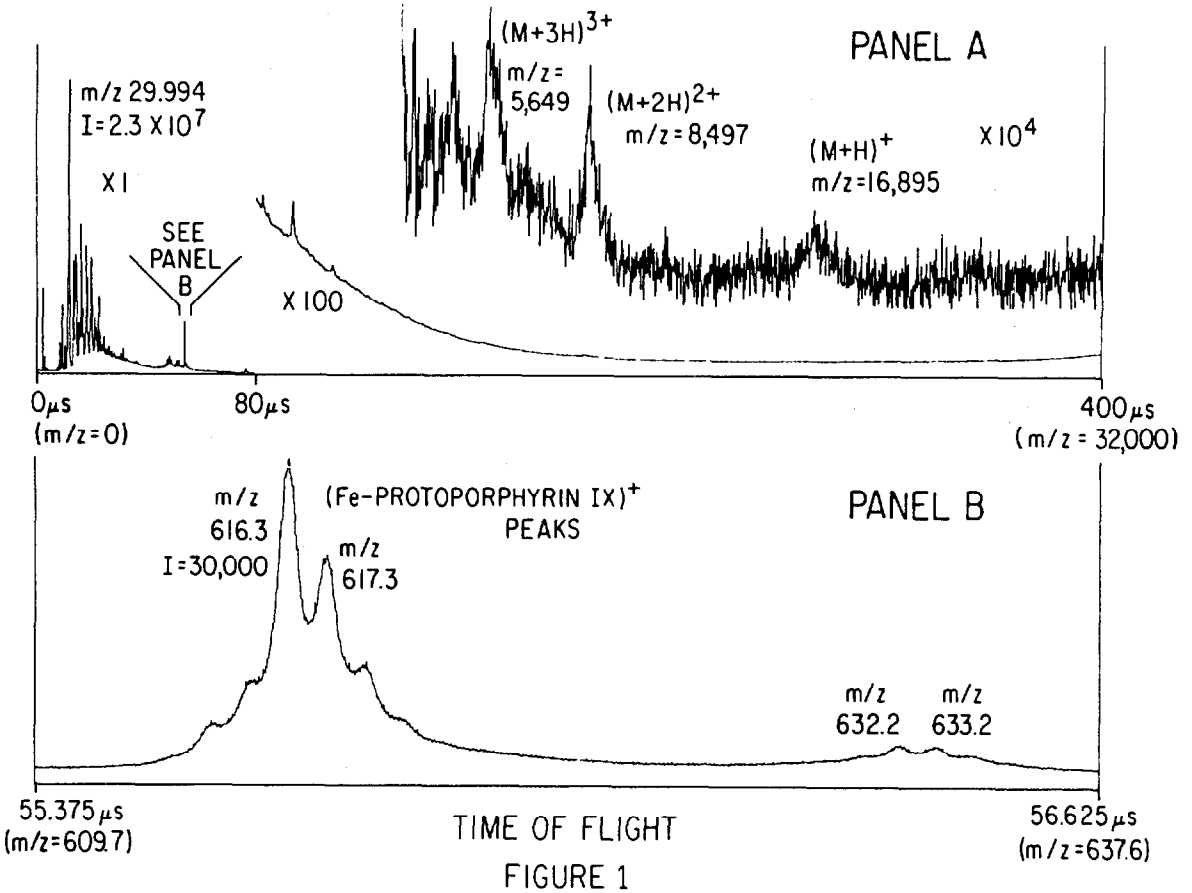

FIGURE 1

The top portion of Figure 1 is a plot of the entire mass spectrum of horse heart myoglobin in the time-of-flight range 0 to 400 μs, which corresponds to a maximum m/z of 32,000. The spectrum has been compressed along the time-of-flight axis so that the data in 640,000 5/8 ns channels in the computer are displayed in 2,000 plot channels. The intensity scale for the section from 80 to 400 μs has been expanded by a factor of 100 to make some relatively small peaks and the background continuum more clearly visible. The trace above this portion of the spectum is obtained by subtracting the smooth continuum underlying the various peaks and magnifying the resulting intensities by a factor of 10^4.

Peaks are observed at m/z 5,649, 8,497 and 16,895, which correspond to $(M+3H)^{3+}$, $(M+2H)^{2+}$ and $(M+H)^{+}$, where M represents the globin portion of the myoglobin. From these m/z values we calculate molecular weight values for the globin of 16,944 u, 16,992 u and 16,894 u, respectively. The simple average of these values is 16,943 u, which agrees well with the formula weight. The bottom section of Figure 1 is a plot of the mass spectrum in the time range 55.375 u to 56.625 u, expanded to show the full time resolution of the system. The peaks at m/z 616.3 and m/z 617.3 are the singly charged iron-protoporphyrin IX and a heavy isotope peak. The peaks at m/z 632 and m/z 633 indicate the possibility of an oxidation product of the iron-protoporphyrin IX.

Without the large storage capability of the MicroVAX I, it would be impossible to observe the molecular ion of the globin and the detail of the iron-protoporphyrin IX peak simultaneously. The system which the MicroVAX I replaced could store only 18,000 channels, so that in order to scan to 400 μs using the old system, the time channels would have had to be approximately 22 ns wide. Using such wide time channels would have obliterated details in the region of the iron-protoporphyrin IX peak.

The fact that the entire spectrum is stored at 5/8 ns resolution also enables one to choose high resolution mass calibration peaks in any region of the mass spectrum without having to arrange for their special handling during data acquisition. By contrast, in the old system, time-of-flight windows were chosen which would encompass specifically chosen calibration peaks, and any flight times which fell within those windows were associated with a special area of computer memory in which the calibration peaks were stored at 5/8 ns resolution. With the MicroVAX I, this more complicated procedure is no longer necessary, and any mass may be chosen as a calibration peak.

b. Dynamic range: Each channel in the time-of-flight spectrum occupies a word in computer memory. The dynamic range of the system is determined by the size of these words. In the MicroVAX I, the 32-bit signed integer word gives a dynamic range of $0\text{-}2 \times 10^9$ counts per time channel. Fig. 1 illustrates the large dynamic range. The intensity of the most intense peak is 2.3×10^7, while that of the $(M+H)^{+}$ peak is 6.2×10^2.

c. Speed: Using an oscilloscope to examine the TDC control lines during a test run, we found that the computer can read a single ion flight time from the TDC and signal that it is ready to accept another in 16.0 μs. When more than one flight time is in the TDC buffer, 19.5 μs are required to transfer each time after the first from the buffer to the computer. When there are no more times in the buffer, the computer rearms the timer for a new scan 18.0 μs after the last transfer. Therefore, the total time required to read flight times from the TDC for n times is $16.0 + ((n - 1) \times 19.5) + 18.0$ μs.

The overall rate at which data can be produced and collected is determined by the sum of the maximum flight time set by the machine operator (the flight period) and the reading time. The actual processing of the times in the computer is done during the next flight period and therefore does not affect the overall speed at which the system can be run, except at very short flight times and/or large multiplicities of ionization, which are not encountered often in practical analyses.

d. Mass accuracy and reproducibility of results: We evaluated the performance of the mass spectrometer with the new data system with respect to mass accuracy and reproducibility by obtaining a series of spectra from human insulin, leu-enkephalin, and crystal violet. Mass calibration was achieved by using the known masses and measured flight times of the H^+ and Na^+ ions to find values for the constants k_1 and k_2 in the equation

$$t = k_1\sqrt{m} + k_2 \qquad (1)$$

where t is the ion flight time in μs, m is the ion mass in u, k_1 is a constant combining the acceleration voltage and flight path, and k_2 is an empirical constant to account for unknown instrument effects.

Because H^+ is desorbed with considerable excess energy, one must correct its mass in order to have an accurate calibration. Such a correction was made by taking a spectrum of crystal violet, which yields a sharp peak at m/z 372.244. We used the peaks at m/z 372.244 and the Na^+ peak at m/z 22.990 to yield values of k_1 and k_2 in equation 1. Using the measured flight time for the H^+ ion in the resulting equation, a corrected m/z value was obtained for H^+. Once the m/z value for H^+ was calculated, it was stored in the data handling program and used in all subsequent calibrations.

Table 1 shows a set of measured m/z values for the $(M+H)^+$ and $(M+2H)^{2+}$ ions from human insulin. Because the isotopic components in these peaks are not resolved, the measurements provide isotopically averaged m/z values. The data in Table 1 were obtained from five separate human insulin samples. Data were collected for one hour for each sample. The standard deviations shown are standard deviations of a single measurement from the mean, and good reproducibility of the data is to be observed. The measured m/z values also agree quite well with the formula weights.

Table 1

Five Independent Measurements of the m/z Values for the $(M+2H)^{2+}$ and $(M+H)^+$ Ions from Insulin Adsorbed on Nitrocellulose

	Measured m/z value of $(M+H)^+$ ion	Δ^a	Measured m/z value of $(M+2H)^{2+}$ ion	Δ^a
Measurement 1	5808.1	-0.5	2904.7	0.1
Measurement 2	5808.2	-0.4	2905.1	+0.3
Measurement 3	5807.2	-1.4	2904.5	-0.3
Measurement 4	5808.6	0.0	2904.8	0.0
Measurement 5	5807.5	-1.1	2904.9	+0.1
Mean	5807.9	-0.7	2904.8	0.0
	$\sigma = 0.06^b$		$\sigma = 0.2^b$	

a. Denotes the absolute deviation of the measured m/z value from the value based on formula weight. These are: $(M+2H)^{2+}$, 2904.8; $(M+H)^+$, 5808.6.

b. σ = standard deviation

In similar fashion to the human insulin measurements, five spectra each were obtained for leu-enkephalin and crystal violet. For the leu-enkephalin, m/z values were measured for the $(M+Na)^+$ ion, calculated m/z 578.259, and the $(M+2Na-H)^+$ ion, calculated m/z 600.241. The mean of the measured values for $(M+Na)^+$ was 578.282, σ = 0.019. For $(M+2Na-H)^+$ the mean was 600.309, σ = 0.011. For crystal violet, m/z was measured for the $(M-Cl)^+$ ion, calculated m/z 372.244. The mean of the measured values was 372.240, σ = 0.005. These results show a mass accuracy of the order of 100 ppm, with good reproducibility.

CONCLUSION

The data system described allows acquisition of data without degrading the inherent resolution of the mass spectrometer, and the system can be built using components that are commercially available. Its main advantage is its large data storage capability. Such capability allows one to examine fine details in all regions of the mass spectrum simultaneously. Another result of this storage capacity is that high resolution calibration peaks can be chosen in any region of the mass spectrum without requiring special treatment during acquisition.

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